Contractor: BIONETICS RESEARCH LABORATORIES, INC.

Contract No.: DA 18-108-AMC-119(A)

FIFTH QUARTERLY PROGRESS REPORT

Covering the Period

23 March 1964 - 22 June 1964

Title: New Methods Development for Irritant Screening

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INTRODUCTION

This fifth quarterly progress report presents a statement of work accomplished during the period from 23 March through 22 June 1964 by Bionetics Research Laboratories, Inc., under Contract No. DA 18-108-AMC-119(A) as amended.

This report is divided into two principal sections, corresponding to the sections of the Contract Statement of Work. Thus, Section I "New Methods Development" discusses the progress which has been made in that phase of the work. Section II "Assessment of Irritant Potentials" discusses the screening operation under the corresponding phase of the work.

SECTION I - NEW METHODS DEVELOPMENT

A. Work Accomplished

Progress continues to be made on various types of isolated tissues, specifically aortic strips from rabbits and segments of the ileum of guinea pigs. The objective of the study is to evaluate various types of isolated tissues and to determine their suitability as biological indicators of irritant action for screening purposes. Because the exact methods used in preparing, handling and studying isolated tissues can have a significant influence on their responsiveness, the full details of the methods used in this study have been incorporated in an appendix. The essential points appear to be that the tissue is removed from the animal promptly after sacrifice, kept in a bicarbonate-buffered glucose containing balanced ionic solution, and, when actually under study, is in equilibrium with a gas mixture containing 5% CO_2 and 95% O_2.

Any study of this type encounters a major difficulty when the compounds to be evaluated are not adequately soluble in water. In theory, stable suspensions should be useful since the tissue would be directly exposed to the suspended particles. In practice, it is difficult to prepare stable suspensions that have little use. The commonly employed device of preparing a suspension with the use of an intermediate solvent has only limited application to the present situation since only small amounts of nonaqueous solvents can be tolerated in the fluid.

Whatever the explanation, the fact remains that none of the irritants studied in this phase of the work produced a measurable contraction of either aortic strips or segments of the ileum. The experimental design incorporated exposure of the muscle strips to histamine, both before and after exposure to the irritants in order to demonstrate that the muscle could contract when exposed to a proper stimulus and to evaluate any possible permanent effects of the irritant. In the course of these studies it appeared that in many instances the response to histamine was less after exposure to the

irritant than before. Consequently, the procedure was modified in an attempt to quantify this alteration of responsiveness to histamine. Most of the irritants studied proved capable of modifying histamine action. However, the concentration in which this effect was observed was highly erratic and was not reproducible.

We must reluctantly conclude that neither the direct response of isolated aortic or ileal smooth muscle to irritants, nor the modification of responsiveness to histamine induced in these tissues by exposure to irritants, constitutes a usable biological index of irritant action worthy of further consideration as a screening procedure.

Species Survey

Work is continuing on the planned survey of cats and monkeys to determine their suitability for irritant testing. The plan of this work has not been altered. It still includes testing of each of several irritants and candidate compounds (covering a range of potency) on each of several individuals in both species. Accomplishment of this complete survey is a time consuming task, since each individual must be allowed to recover from whatever effects are produced in order that a subsequent exposure may be made to another compound.

At the present stage no individual has received all compounds. Consequently, at this point no reliable conclusions can be drawn. As previously stated it seems wiser to defer discussion of results of this study until the complete series has been accomplished.

Aerosol Exposures

Equipment has been assembled for the performance of a relatively crude type of exposure of small rodents (mice) to aerosols of irritants. Evaluation of this as a possible screening technique was effectively begun only very late in the report period. The number of exposures accomplished to date is completely inadequate to warrant publication of conclusions in this report. The animals do appear to show irritant effects over and above those caused by the solvents necessary for aerosol generation, however, conclusions as to relative activity of different compounds cannot be drawn at this time.

B. Plans for Subsequent Period

The evaluation of isolated tissues as biological indicators of irritant action suitable for screening procedures is considered completed. Neither rabbit aortic strips nor guinea pig ileum appear useful. Investigation of other tissues does not seem warranted.

Investigation is proceeding of a crude aerosol exposure technique. Conclusions as to the utility of this procedure can be expected by the end of the next reporting period.

The multiple cross-over species survey is continuing. Completion of this study is anticipated within the next reporting period.

SECTION II - ASSESSMENT OF IRRITANT POTENTIAL

Accomplishments under this section of the Contract reflect the changes in procedure effective 13 May by modification No. 3 of the basic contract. The essence of the modification is to require certification by a certified pathologist or a doctor of veterinary medicine that whatever effects have been produced by the application of an irritant to rabbit's eyes or skin have disappeared within the period of observation and further, to retest all compounds previously studied under this Contract for which such certification was not acquired. A limited number of compounds have been deleted from the retest instructions on the authority of the Contract Project Officer. The decision to delete the compounds was based on the premise that they are too irritant to warrant further study.

Coincident with these changes in objectives there has been a modification of the procedures to be used in studying skin and eye irritation. In addition, the format of the technical reports has been changed. This latter change is occasioned primarily by initiation of the planned card-punching aspects of the technical reports. As of this writing, approximately 8000 cards have been punched and dispatched to the Contract Project Officer.

The procedural changes with respect to the eye irritant study are insignificant and need not be mentioned further. The procedural changes with respect to the skin irritation study involve a shift from polyethylene glycol to methanol as the solvent in which water insoluble substances are to be studied. This decision was made by the Contract Project Officer and is, we understand, based upon an apparent protective action of polyethylene glycol. This change will make later data imperfectly comparable to data obtained earlier but will not otherwise modify the Contract.

A. Compounds Returned

Returned 1 June 1964

CS 11470	CS 32718	CS 42693	CS 45223
CS 18107	CS 32811	CS 42706	CS 45253
CS 18108	CS 33272	CS 42987	CS 45245
CS 18109	CS 33347	CS 43023	CS 45402
CS 18110	CS 33359	CS 43111	CS 45414
CS 18175	CS 33403	CS 44854	CS 45506
CS 18454	CS 39525	CS 44856	CS 45509
CS 20267	CS 41576	CS 44857	CS 45512
CS 20269	CS 41727	CS 44863	CS 45514
CS 22658	CS 42039	CS 44881	CS 45657
CS 30482	CS 42319	CS 45010	CS 45664
CS 31513	CS 42679	CS 45021	CS 45665
CS 32716			

B. Work Completed

Assessment has now been completed on the following 99 compounds.

EYE AND SKIN

CS 18107	CS 33434	CS 44854	CS 45020
CS 18108	CS 37144	CS 44855	CS 45021
CS 18109	CS 39523	CS 44856	CS 45223
CS 18110	CS 39525	CS 44859	CS 45246
CS 18175	CS 42296	CS 44860	CS 45404
CS 18454	CS 42310	CS 44861	CS 45405
CS 20267	CS 42312	CS 44863	CS 45408
CS 20269	CS 42315	CS 44864	CS 45410
CS 22656	CS 42319	CS 44881	CS 45414
CS 22658	CS 42642	CS 44882	CS 45429
CS 30482	CS 42647	CS 44883	CS 45443
CS 31326	CS 42650	CS 44884	CS 45508
CS 31513	CS 42663	CS 44885	CS 45509
CS 32718	CS 42738	CS 44886	CS 45510
CS 32811	CS 43029	CS 44887	CS 45511
CS 33225	CS 43058	CS 44889	CS 45512
CS 33260	CS 43608	CS 44891	CS 45513
CS 33272	CS 43807	CS 44938	CS 45514
CS 33326	CS 43982	CS 45003	CS 45641
CS 33335	CS 44475	CS 45006	CS 45646
CS 33347	CS 44483	CS 45010	CS 45651
CS 33359	CS 44523	CS 45013	CS 45652
CS 33360	CS 44530	CS 45014	CS 45664
CS 33403	CS 44548	CS 45015	CS 47753
CS 33410	CS 44851	CS 45018	

Compound on which assessment is incomplete (evaluated for skin irritation only).

SKIN

CS 43146

Compound on which assessment is incomplete (evaluated for eye irritation only).

EYE

CS 29338

C. Future Plans

Work under this section of the Contract is progressing smoothly and no difficulty is anticipated in completion of the scheduled list of compounds within the period of the Contract.

SECTION III - SUMMARY

1. Rabbit aorta and guinea pig ileum were evaluated to determine their suitability as biological indicators of irritant action for screening purposes.

2. The effects of irritants on incised aortic and ileac tissue indicate that it is not deemed advisable to further consider them as biological indicators.

3. Cats and monkeys are being evaluated for irritant screening.

4. A study is being made of a crude aerosol exposure technique.

APPENDIX

METHODS FOR STUDY OF ISOLATED TISSUES

Aortic Strip

A rabbit was sacrificed by a blow on the head. The thoracic aorta was immediately removed and placed in warm bicarbonate-buffered, glucose-containing balanced ionic solution of the composition given in the accompanying table. The lumen of the aorta was washed gently to remove blood and fat. The connective tissue was carefully dissected away. A spiral cut was made from left to right producing a strip approximately 3 mm wide and 3 cm long. The strip was kept moist until used. The strip was then placed in an isolated organ bath (maintained at 37°C, aerated with a mixture of 95% O_2 and 5% CO_2) connected at the bottom to the glass air tube and at the top to an ink-writing lever. The magnification of the lever was approximately 20:1 and the load was 2.5 gm.

Vibration was applied to the supporting stand throughout the experiment in order to reduce the friction of the writing point and to obtain the maximum possible response. In order to establish a baseline, the aortic strip was allowed to relax for 30 to 60 minutes before the application of irritants or standards. The aortic strip was then permitted to react to an irritant for three minutes. Thereafter the bath was rinsed three times and the strip gently stretched until the lever rested at the baseline. Irritants were added at seven-minute intervals. Modification of response to histamine was studied by incorporating the desired amount of the irritant into the bathing solution and determining a dose-response curve by addition of varying amounts of histamine.

SOLUTION FOR AORTIC STRIP

	mg/ml
KCl	0.35
$CACL_2 \cdot 2H_2O$	0.37
KH_2PO_4	0.16
$MgSO_4 \cdot 7H_2O$	0.29
NaCl	6.9
$NaHCO_3$	2.8
Glucose	1.8

Guinea Pig Ileum

A guinea pig (300 to 500 gm body weight) was sacrificed by a blow on the head. The terminal portion of the ileum was removed as quickly as possible and placed in a bicarbonate-buffered, glucose-containing balanced ionic solution of the composition given in the accompanying table. The gut was washed through several times to remove feces. Connective tissue was carefully dissected away. The ileum was kept moist until use. A 3 cm long segment was suspended in a tissue bath between the air tube at the bottom and the writing lever at the top in such a way that both ends of the segment remained open. A gas mixture of 95% O_2 and 5% CO_2 was bubbled through the solution in the tissue bath. Contractions were recorded with an ink-writing lever having a magnification of approximately 10:1 and imposing a 1.0 gm load.

To obtain the maximum possible response vibration was applied to the supporting stand to minimize friction of the writing point. In order to establish the baseline before starting the experiment, the ileum was allowed to relax for 30 to 60 minutes. Added drugs were permitted to act for 30 seconds following which the gut was washed three times by overflow. During the wash-out, the ink lever returned to its original baseline. Drugs were added at four-minute intervals. Modification of response to histamine was studied either by incorporating the desired amount of irritant into the bathing solution and determining the dose response curve by addition of varying amounts of histamine, or by simultaneous (rapid sequential) addition of irritant and histamine.

SOLUTION FOR ILEUM

	mg/ml
KCl	0.35
$CaCl_2 \cdot 2H_2O$	0.48
KH_2PO_4	0.16
$MgSO_4 \cdot 7H_2O$	0.60
NaCl	6.60
$NaHCO_3$	2.10
Dextrose	2.07